REMARKS/ARGUMENTS

I. Status of the Claims

Claims 58 and 65 have been amended in view of a reference submitted in Applicants' Supplemental Information Disclosure Statement, Rust, et al. (Am. J. Resp. Cell. Mol. Biol. 14:121-130 (1996), herein "Rust"). Applicants have provided, for the Examiner's convenience, a schematic showing the cloning vector pGEM (Attachment A). Applicants have amended claims 58 and 65 to recite that both promoters function in a eukaryotic cell. A selectable marker on the pGEM vector, the Amp gene, is transcribed by its own promoter, a prokaryotic promoter. Support for this amendment is found, *inter alia*, in the specification on pages 76-78, the section that describes polyadenylation trap vectors and page 81, the section that describes dual polyadenylation trap/splice acceptor trap vectors. The promoters would both inherently function in a eukaryotic cell.

Claims 102 and 103 have been amended to recite that the genomic DNA and vector DNA are introduced into the cell in a ligated or unligated form. Support for this amendment can be found *inter alia* in the specification on page 105, lines 22-24, and page 103, lines 9 and 10. Claims 106 and 107 have been amended to recite that the method is practiced in a cell in which splicing can occur. Support for this amendment can be found *inter alia* in the specification on page 106, lines 22-24.

II. The Rejections

A. Rejection Under 35 U.S.C. § 112, First Paragraph

On page 2 of the Office Action, claims 98-99 and 115-116 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that they are not enabled. Applicants respectfully traverse the rejection.

The Examiner states that claims 98-99 are not enabled because the vector of claim 97 cannot express the first selectable marker when the vector integrates into the genome and the method requires the cells to express both the first and second selectable marker sequence. Applicants direct the Examiner to A. and B. in Appendix A and explain as follows. When the vector (in either configuration) integrates upstream from a *single exon gene* (A. and B.), there is no splicing because there is no splice acceptor in a single exon gene. Therefore, both selectable markers are expressed in active form. In contrast, when the vector (in either configuration) integrates upstream of a *multi-exon gene* (C. and D.), any exon downstream from the first exon in the gene will contain a splice acceptor. Splicing will occur from the splice donor on the vector to the splice acceptor on the exon (exon II through the last exon) and, when splicing occurs, the first selectable marker is not expressed in active form. Accordingly, for single exon genes two selectable markers are active. This activity indicates that the vector has integrated upstream from a single exon gene.

With respect to claims 115 and 116, the principle is the same as for A., B., C. and D.. With a single exon gene, both markers will be expressed because there is no splicing. If the vector integrates upstream of a multi-exon gene, splicing will occur. When splicing occurs the negative marker is spliced out. See E. in Appendix A. This provides a way to know if the vector has integrated upstream of a single exon gene.

The Examiner asks how the positive marker can be expressed when splicing occurs where the negative selectable marker is located upstream of the positive marker. See E. in Appendix A. The positive selectable marker is driven by its own promoter. Therefore, transcription of the positive marker is unaffected by splicing.

On page 4 of the Office Action, claims 101-103 and 105-109 are rejected as not being enabled. With respect to claim 101, the Examiner states that the specification does not show how to make and use a transcript that contains exon I of a multi-exon gene. The Examiner indicates that the presence of an unpaired splice donor site would cause exon I to be spliced out and, therefore, a transcript containing exon I can not be obtained. Please see D. in Appendix A. Applicants explain as follows. The presence of independent promoters provides for transcription of exon I from a multi-exon gene. Splicing out the negative marker indicates whether the activated gene is a single exon or multi-exon gene.

Claims 102 and 103 are rejected on the grounds that the specification does not show how to combine the vectors with isolated genomic DNA in any manner without inserting the vectors into the genomic DNA. Applicants point out that vector and genomic DNA can be introduced

into the cell as covalently ligated or unligated forms. Co-transformation of isolated DNA fragments results in ligation in the cell. This was known to the person of ordinary skill in the art. Applicants have amended the claim to clearly point out the process.

Claims 106 and 107 are rejected on the grounds that the specification does not enable practicing the methods in bacterial cells with the bacterial genome because there are no splicing sequences in the bacterial genome. Accordingly, the claims have been amended to recite that the method is practiced in a cell in which splicing can occur. Applicants point out that, although native prokaryotes do not contain splicing enzymes and DNA which is normally spliced, it is possible to transfect prokaryotic cells with eukaryotic DNA and transfect the cells with genes that express splicing enzymes. In this way, using Applicants' vectors and isolated genomic DNA, the method could potentially be practiced in cells that do not normally regulate their own genes by splicing.

In view of the above discussion and amendments to the claims, Applicants submit that all grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

B. Rejection Under 35 U.S.C. § 112, Second Paragraph

On page 9 of the Office Action, claims 95 and 96 have been rejected on the grounds that they do not particularly point out the subject matter of the invention. Applicants respectfully traverse the rejection.

The Examiner indicates that the term "said cDNA" is unclear. Accordingly, Applicants have amended the claims to recite that the cDNA refers to cDNA in the appropriate step.

C. Rejection Under 35 U.S.C. § 102

1. Duyk

On page 9 of the Office Action, the Examiner has rejected claims 58, 61, 62, 65, 74, 77-79, 82, 84-87, 90, 92 and 102 under 35 U.S.C. § 102(b) for being anticipated by Duyk et al. (Proc. Nat'l. Acad. of Sci., USA 87:8995-8999 (1990), herein "Duyk"). Applicants respectfully traverse the rejection.

Duyk does not meet all the limitations of the claims. Both LTRs in the Duyk vector contain functional polyadenylation signals. Accordingly, there is a functional polyadenylation signal downstream from the Neo gene. Applicants also point out that the CAT gene is transcribed in a direction opposite from both the Neo gene and the globin exon sequence. Accordingly, this reference does not anticipate any of the claims as previously pending.

In view of Applicants' argument, Applicants submit that Duyk does not anticipate independent claims 58 and 65 and, therefore, would not anticipate any of the dependent claims. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

2. Treco

On page 11 of the Office Action, claims 58, 61-65, 73, 76, 78-79, 81, 84-87, 89 and 92 have been rejected under 35 U.S.C. § 102(e) on the grounds that they are anticipated by Treco, et al. (U.S. Patent No. 6, 270,989, herein "Treco"). Applicants respectfully traverse the rejection.

The Examiner cites the targeting construct pRTPO1. But pRTPO1 contains a polyadenylation signal operably-linked to the DHFR and Neo gene. Accordingly, this vector does not anticipate claims 58 and 65 as previously pending. Since it does not anticipate these independent claims, it does not anticipate any of the claims that depend from claims 58 and 65. Applicants provide the Examiner with a schematic of the orientation and components in pRTPO1 (Attachment B).

In view of the above argument Applicants submit that the grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

3. Hay

On page 11 of the Office Action, claims 58, 61, 62, 65, 72, 78-80 and 84-86 have been rejected under 35 U.S.C. § 102(e) [sic] as being anticipated by Hay, et al. (Proc. Natl. Acad. Sci. 94:5195-5200 (1997), herein "Hay").

Hay teaches a selectable marker (Amp) with its native promoter and a promoter operably-linked to a splice donor sequence. The Amp gene is designed for selection in a prokaryotic organism and, accordingly, has a prokaryotic native promoter. Claims 58 and 65, however, have been amended to recite that both promoters function in a eukaryotic cell. Accordingly, Hay does not anticipate the claims as amended.

In view of the above amendment Applicants submit that the grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

Accordingly, Applicants believe that the application is in condition for allowance. Early notification in that regard is requested. If the Examiner believes that a telephonic interview would expedite prosecution of this case, he is invited to contact Applicants' attorney, Anne Brown, at 216-426-3586 or Cynthia L. Kanik, Ph.D., at 617-227-7400.

The Commissioner is hereby authorized to charge any fee deficiency to Deposit Account No. **50-2546**, referencing Attorney Docket No. **ATX-007CP4DV7**.

Respectfully submitted,

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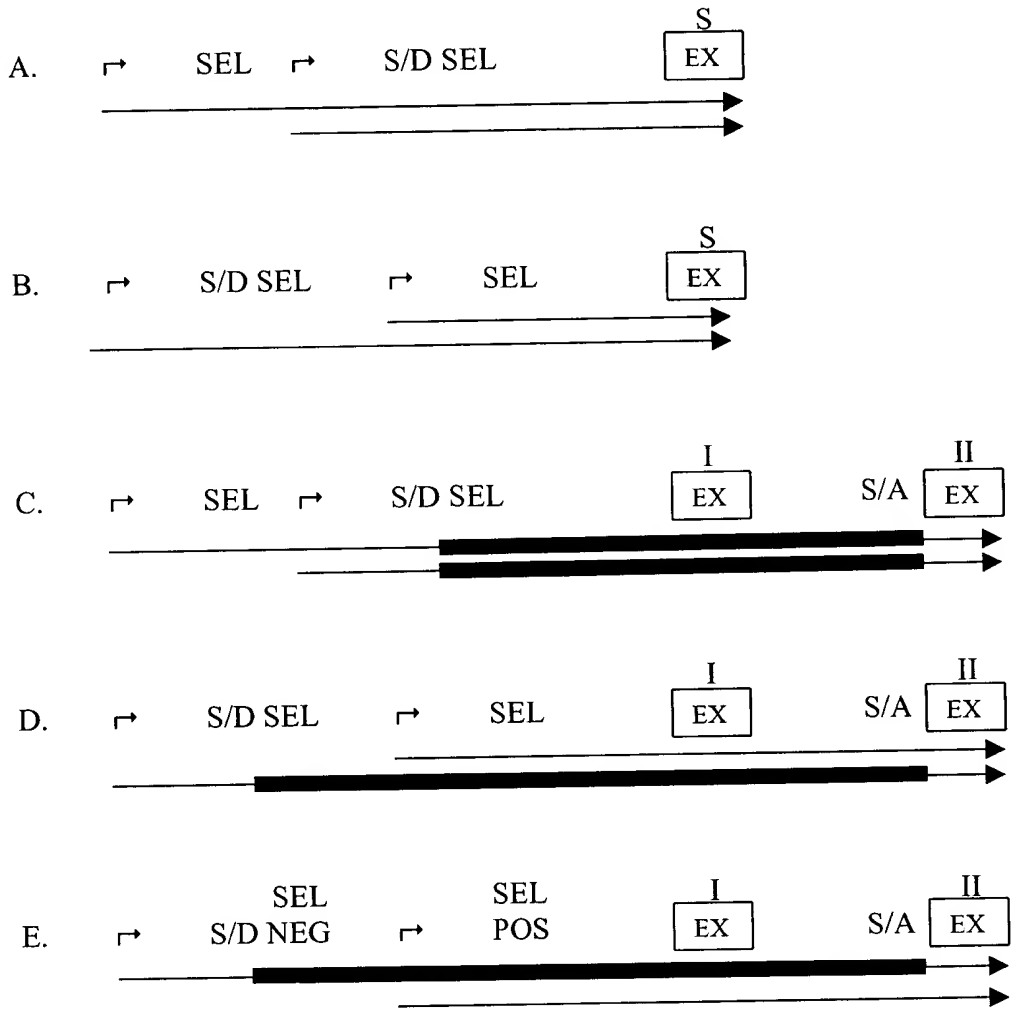
APPENDIX A



▬	box	= spliced out
↱		= promoter
→		= transcript
S EX		= single exon
SEL		= selectable marker
S/D		= splice donor
S/A		= splice acceptor
I		= exon I
II		= exon II

ATTACHMENT "A"

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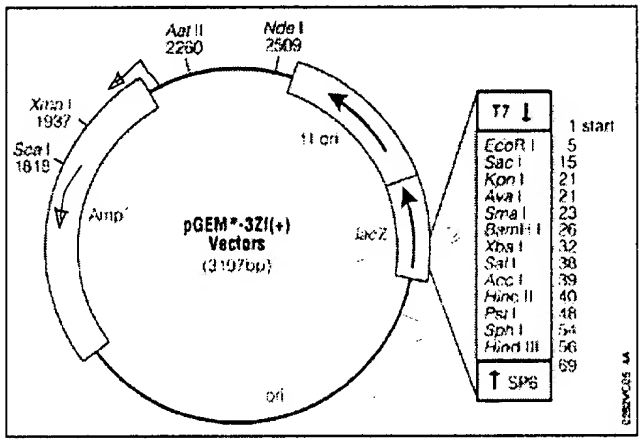

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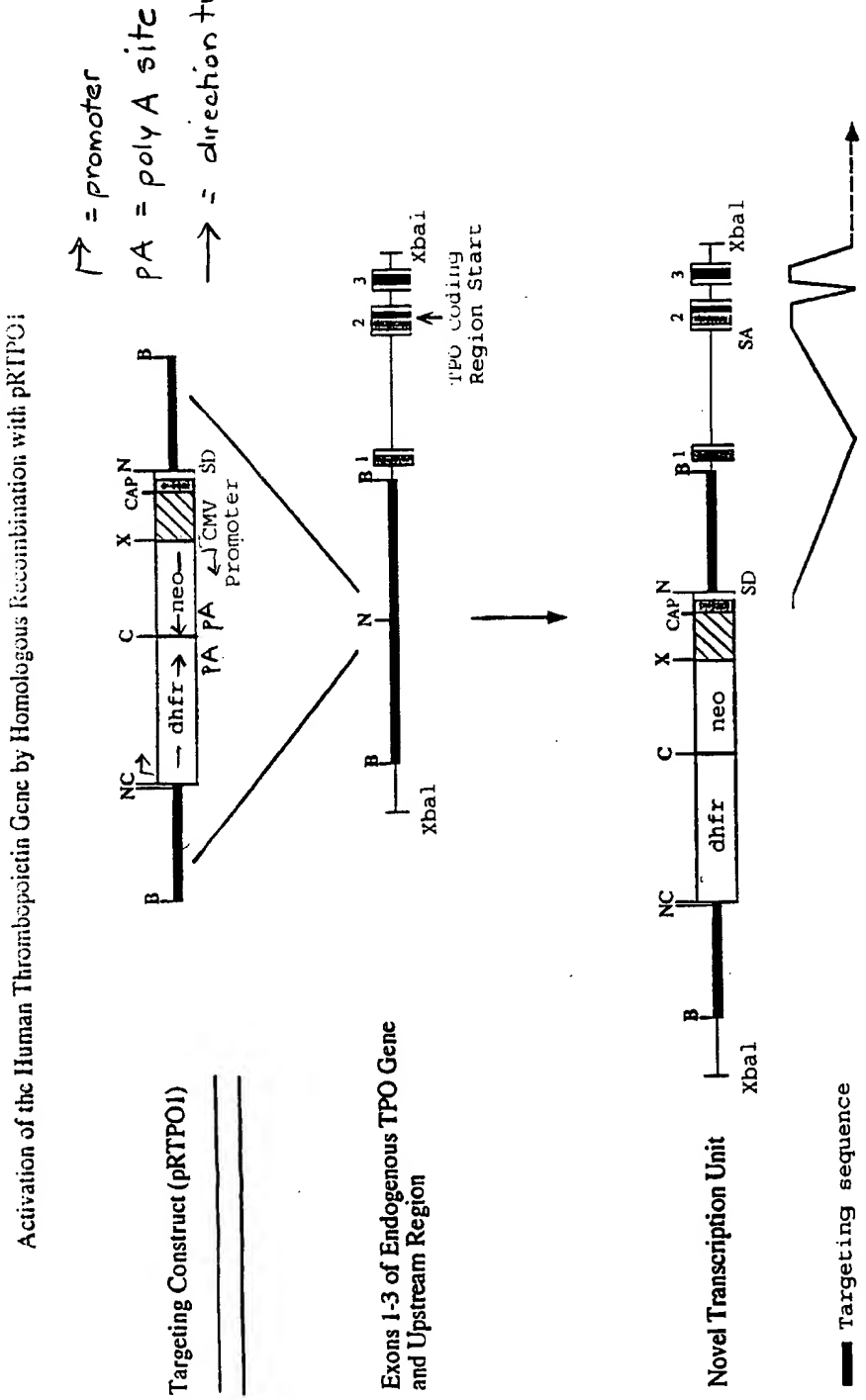

FIG. 6